

EFFECT OF HOT WATER PRETREATMENT SEVERITY ON THE DEGRADATION AND ENZYMATIC HYDROLYSIS OF CORN STOVER

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ABSTRACT. The effects of hot water pretreatment on the degradation and enzymatic hydrolysis of corn stover were studied. Nearly 100% cellulose recovery in the solid fraction was obtained when corn stover was pretreated at 170 °C to 210 °C for 3 to 10 min. The highest pretreatment severity of 4.239 (210 °C, 10 min) resulted in the highest solid solubilization (37.0%) and xylan solubilization (90.5%). At this severity, inhibitors such as acetic acid, furfural, and hydroxymethyl furfural (HMF) also reached the highest levels of 7.1, 4.6, and 0.6 g L⁻¹, respectively. When the pretreatment temperature was less than 190 °C, the furfural concentration was below 1.0 g L⁻¹ and no significant levels of HMF were detected. Enzymatic hydrolysis results showed that increased glucose yields were obtained with increased pretreatment temperatures of corn stover. The highest glucose yield of 89.2% was obtained at the pretreatment severity of 3.716 (210 °C, 3 min). Due to the degradation of sugars, a glucose yield of 85.9% was obtained at the highest pretreatment severity of 4.239 (210 °C, 10 min).

Keywords. Corn stover, Enzymatic hydrolysis, Liquid hot water, Pretreatment.

Surging demand for fossil fuels brings economic problems (e.g., trade deficit), environmental problems (e.g., greenhouse gas emissions), and social sustainability problems (e.g., energy security) (Lau and Dale, 2009). Development of renewable energy as an alternative to petroleum-dependent energy is now a high priority in many countries (Galbe and Zacchi, 2007). Lignocellulosic biomass, such as agricultural and forestry residue, is the most promising feedstock for sustainable production of transportation fuels and chemicals due to its sufficient supply. However, lignocellulosic biomass is a heterogeneous lignin carbohydrate complex (LCC), resistant to biological or chemical catalysts for industrial fermentation (Himmel et al., 2007). Pretreatment is an essential step for bioconversion of lignocellulosic biomass as it significantly reduces the recalcitrance of biomass via breaking lignin seals and disrupting the crystalline structure of cellulose (Mosier et al., 2005a; Wyman et al., 2005).

Studies have shown that liquid hot water (LHW) pretreatment significantly enhanced enzymatic hydrolysis of various

lignocellulosic materials, such as corn stover (Mosier et al., 2005b), corn fiber (Weil et al., 1998a), and hybrid poplar (Kim et al., 2009). LHW pretreatment is generally conducted at elevated temperatures (120 °C to 260 °C) for 5 to 15 min under pressure without addition of base or acid (Kim et al., 2009; Weil et al., 1998b). Organic acids generated during LHW pretreatment are believed to act as catalysts for the hydrolysis of holocellulose (cellulose and hemicelluloses) to soluble oligo- and monosaccharides. However, accumulation of organic acids at severe pretreatment conditions has caused the degradation of monosaccharides to byproducts (e.g., hydroxymethyl furfural (HMF), furfural, formic acid, and levulinic acid) (Weil et al., 1998b). These degradation products are inhibitory to ethanol-producing microorganisms. LHW pretreatment at controlled pH has been effective in minimizing the degradation of monomeric sugars (Weil et al., 1998b; Mosier et al., 2005b). For pH-controlled LHW pretreatment, a base, such as potassium hydroxide, must be added to keep the pH within a range of 5 to 7 (Weil et al., 1998b). Without the addition of a base, the pH during pretreatment is generally within 4 to 5 (Kim et al., 2009).

Temperature and time are two critical components of LHW pretreatment. Pretreatment severity (R_0) was defined by Overend and Chornet (1987) as a function of temperature (T , °C) and holding time (t , min):

$$R_0 = t \times \exp((T - 100)/14.75) \quad (1)$$

This relationship has been used to determine treatment intensity in a given LHW pretreatment process. Several studies also included pretreatment pH for a modified definition of severity: $R'_0 = [H^+]R_0$. The effects of pretreatment severity on the enzymatic hydrolysis of wheat straw (Peterson et al., 2009), rye straw (Rogalinski et al., 2008), corn stover (Yang and Wyman, 2004), and rice straw (Yu et al., 2010) have been reported in the literature. Although pretreatment severity enables easy comparison of pretreatment results, the correla-

Submitted for review in April 2010 as manuscript number BE 8509; approved for publication by the Biological Engineering Division of ASABE in October 2010.

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tions between pretreatment severity and its effects are difficult to establish (Kabel et al., 2007; Perdersen and Meyer, 2010; Rogalinski et al., 2008).

The aim of this study was to evaluate the effect of LHW pretreatment severity on the degradation and subsequent enzymatic hydrolysis of corn stover. In addition, the effects of pretreatment temperature and time were compared to that of pretreatment severity. This study enabled us to clarify how pretreatment severity represented the combined effects of pretreatment temperature and time.

MATERIALS AND METHODS

MATERIALS

Corn stover was collected from farm at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, Ohio. It was dried at 40°C, ground to pass a 3/16 in. (4.8 mm) screen, and stored in an airtight container at room temperature prior to use. The raw corn stover was composed of 39.4% cellulose, 18.4% xylan, and 20.2% lignin. Spezyme CP obtained from Genencor (Palo Alto, Cal.) was used in the enzymatic hydrolysis tests. The activity of Spezyme CP was determined to be 50 FPU mL⁻¹ according to NREL Laboratory Analytical Procedure (LAP) 006 (Adney and Baker, 1996).

LHW PRETREATMENT

A one-liter Parr reactor (Parr Instrument Co., Moline, Ill.) was used for the LHW pretreatment. Forty grams of corn stover (dry mass) was evenly mixed with 400 mL of deionized (DI) water in the stainless steel vessel of the reactor. The temperature and pressure of the reactor were automatically controlled. The mixture of corn stover and DI water was heated to 170°C, 180°C, 190°C, 200°C, or 210°C within 40 to 50 min with continuous agitation (approximately 400 rpm). After the mixture was pretreated at that temperature for 3, 5, and 10 min, the vessel was cooled to room temperature (25°C) by immersing the vessel in tap water for approximately 30 min. The pretreated slurry was separated by vacuum filtration using glass fiber filters. The pretreatment liquor was collected for analysis of inhibitory compounds. The solid retained on the filter paper was further washed with 1000 mL of DI water at room temperature for compositional analysis and enzymatic hydrolysis tests.

ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis tests were conducted following NREL protocol (LAP-008; Dowe and McMillan, 2001). Enzyme (Spezyme CP) loading of 20 FPU g⁻¹ solid was used for the enzymatic hydrolysis tests. The enzymatic hydrolysis was conducted on a rotary shaker (Excelsa E24, New Brunswick Scientific, New Brunswick, N.J.) at a temperature of 50°C and an agitation speed of 130 rpm. After 48 h, the hydrolyzed slurry was boiled to terminate the hydrolysis. After cooling, the supernatant was filtered through a 0.2 µm nylon membrane filter for sugar analysis.

ANALYTICAL METHODS

The dry mass and composition of untreated and pretreated corn stover were determined following NREL protocols (Sluiter et al., 2008). The sugar concentrations were determined by high-performance liquid chromatography (HPLC) (1200 Series, Agilent Technologies, Santa Clara, Cal.) with

an Aminex HPX-87P column (BioRad, Inc., Hercules, Cal.) and a refractive index detector (RID). HPLC-grade water was used as the mobile phase at a flow rate of 0.6 mL min⁻¹. The temperatures of the column and detector were maintained at 80°C and 55°C, respectively. The sugar degradation products in the liquor fraction after pretreatment were analyzed by HPLC using a Rezex RFQ Fast Acid column (Phenomex, Inc., Torrance, Cal.). Sulfuric acid at a concentration of 0.005 N was used as the mobile phase with a flow rate of 0.6 mL min⁻¹. The temperatures of the column and detector were 55°C and 45°C, respectively.

Solid or polysaccharide (cellulose or xylan) recovery after pretreatment was defined as:

$$\text{Recovery (\%)} = \frac{M_{\text{recovered}}}{M_{\text{raw}}} \times 100 \quad (2)$$

where M_{raw} represents the amount of solid, cellulose, or xylan in the raw material, and $M_{\text{recovered}}$ represents the amount of solid remaining after pretreatment or the amount of polysaccharide (cellulose or xylan) in the solid after pretreatment.

Sugar (glucose or xylose) yield of enzymatic hydrolysis was defined as:

$$\text{Sugar yield (\%)} = \frac{M_{\text{sugar}}}{M_{\text{polysaccharide}}} \times 100 \quad (3)$$

where M_{sugar} is the amount of glucose or xylose released by enzymatic hydrolysis, $M_{\text{polysaccharide}}$ is the amount of glucan or xylan in the raw materials, and f is the conversion factor from polysaccharides to monomeric sugars (180/162 for glucan to glucose and 150/132 for xylan to xylose (Zeng et al., 2007)).

STATISTICAL ANALYSIS

All data reported are average values of duplicates. A 95% confidence level was used for statistical analysis, and one-way analysis of variance (ANOVA) was performed with Tukey simultaneous tests (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

SOLID AND SUGAR RECOVERY

The solid recovery of corn stover after pretreatment is presented in table 1. Solid recovery was significantly affected by pretreatment temperature. At a pretreatment time of 3 min, the solid recovery decreased from 87.7% to 65.4% when temperature was increased from 170°C to 210°C. Increasing pretreatment time also caused decreases in solid recovery, but the effect was not significant ($p > 0.05$).

Figure 1 shows the total recovery of glucan and xylan in the solid fraction after pretreatment. Glucan recovery in the solids after pretreatment was affected slightly by pretreatment temperature and time. Only 2% to 4% of glucan solubilization was observed at a temperature of 210°C. For all other temperatures, the total glucan recovery in the solid fraction was nearly 100%. Pretreatment time within the range of 3 to 10 min had no significant effect on the glucan recovery ($p > 0.05$). The effect of severity ($\log R_0$) on cellulose recovery was not significant (fig. 1b). The glucan content in the treated solids increased from 39.4% to 61.0% due to the substantial solubilization of xylan. Weil et al. (1998b) also reported that a high cellulose recovery (>98%) and increased cellulose

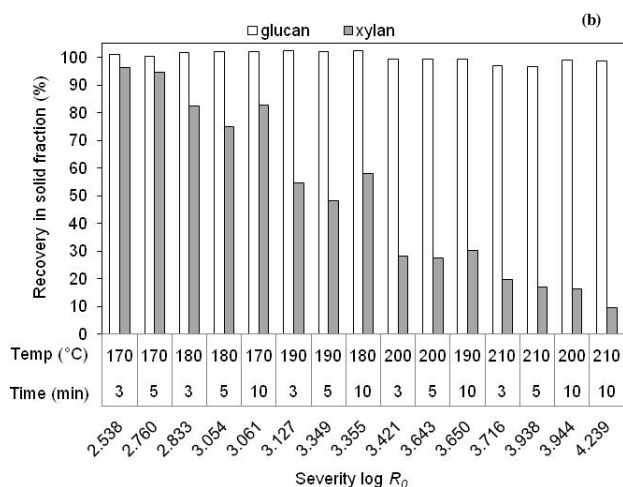
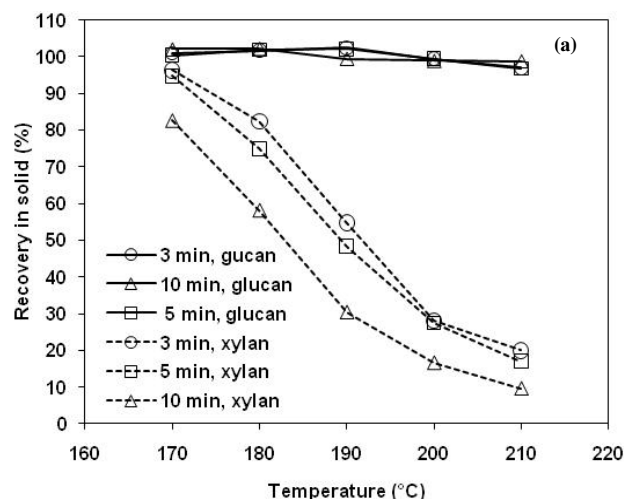


Figure 1. Effect of LHW pretreatment on polysaccharide recovery in the solid fraction: (a) effect of temperature and time, and (b) effect of severity. Blank column is glucan and gray column is xylan.

Table 1. Solid recovery after LHW pretreatment in pretreated solids.

Temperature (°C)	Time (min)	Severity (logR ₀)	Solid Recovery (%)
170	3	2.538	87.7 ±0.6
180	3	2.833	87.7 ±0.4
190	3	3.127	75.1 ±0.4
200	3	3.421	66.5 ±0.1
210	3	3.716	65.4 ±0.5
170	5	2.760	88.9 ±0.5
180	5	3.054	79.6 ±1.4
190	5	3.349	71.2 ±1.3
200	5	3.643	64.7 ±0.3
210	5	3.938	66.0 ±2.3
170	10	3.061	83.7 ±1.4
180	10	3.355	75.6 ±3.8
190	10	3.650	65.2 ±0.6
200	10	3.944	64.0 ±0.7
210	10	4.239	63.0 ±0.4

content in pretreated solids were observed when yellow poplar wood sawdust was pretreated by LHW at temperatures of 220 °C to 240 °C regardless of pH control. However, when the temperature increased to 260 °C, 25% glucan solubilization

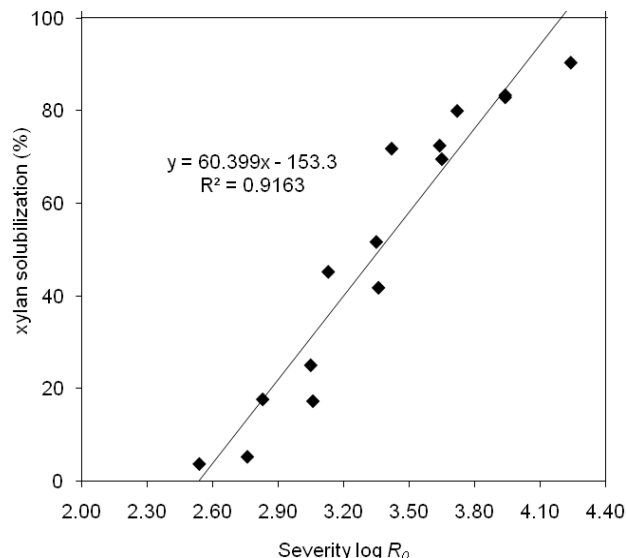


Figure 2. Correlation between xylan solubilization and pretreatment severity.

was observed at a pretreatment temperature of 260 °C with uncontrolled pH, whereas negligible solubilization of glucan occurred with pH control at this temperature. Therefore, an optimal temperature of LHW is important to prevent significant degradation of cellulose and subsequent formation of inhibitors in the liquid fraction.

Hemicellulose solubilization is one of the major effects of LHW pretreatment. As shown in figure 1a, xylan recovery in the pretreated solids decreased with the increase in pretreatment time and temperature. Within the tested temperature range (170 °C to 210 °C), each increase in temperature of 10 °C resulted in a 10% to 30% decrease in xylan recovery. More than 90% of the xylan was solubilized when corn stover was pretreated at 210 °C for 10 min, which corresponded to a low xylan content of 2.8% in the pretreated solids. Liu and Wyman (2003) also showed that xylan removal increased with pretreatment temperature and time, resulting in more than 90% xylan solubilization at 220 °C for 16 min. Nearly complete solubilization of hemicellulose and less than 10% solubilization of cellulose was also observed during LHW pretreatment of other biomass feedstocks, such as sugarcane bagasse and aspen chips at 220 °C (van Walsum et al., 1996). A good correlation ($R^2 = 0.91$) between xylan solubilization and pretreatment severity was also observed (fig. 2). As the severity increased to 4.1 or above, xylan was almost completely solubilized. Petersen et al. (2009) also found that hemicellulose recovery of the fiber fraction decreased from 70% to 30% when pretreatment severity increased from 3.28 to 3.88, respectively, in hydrothermal pretreatment of wheat straw.

FORMATION OF INHIBITORS DURING LHW PRETREATMENT

Formation of Acetic Acid

Acetylation on the xylan backbone has been shown to reduce the accessibility of xylan/cellulose and inhibit the activity of xylanase and debranching enzymes (Kumar et al., 2009). In another study, the removal of acetyl groups from corn stover enhanced enzymatic hydrolysis due to increased cellulose accessibility and enzyme effectiveness (Kumar and Wyman, 2009). As acetylated xylooligomers in the liquid

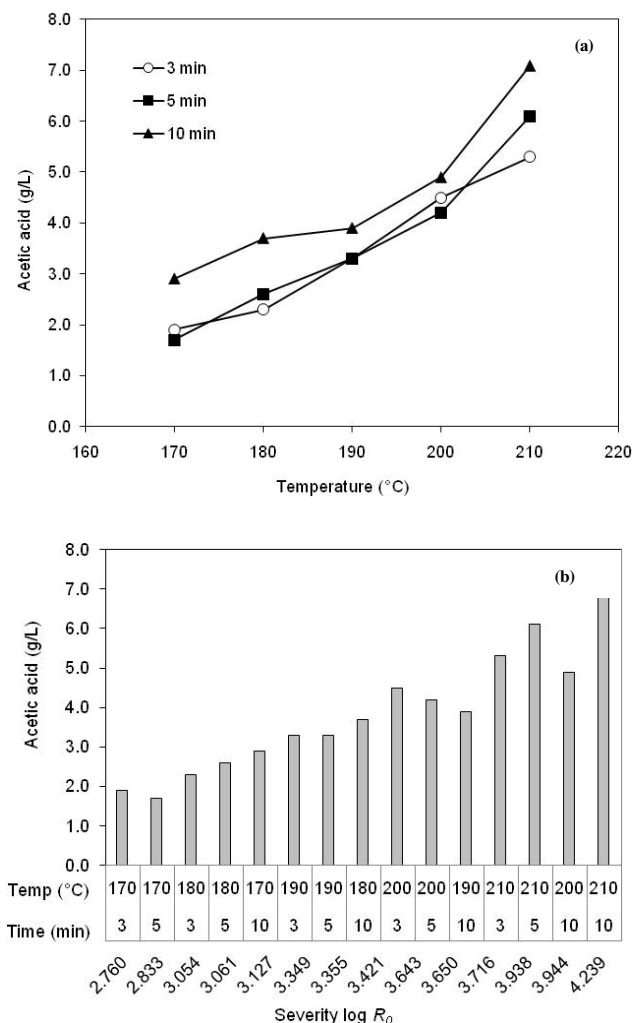


Figure 3. Effect of LHW pretreatment on the formation of acetic acid: (a) effect of temperature and time, and (b) effect of severity.

fraction are believed to strongly inhibit enzymes (Kabel et al., 2007), acetyl groups are preferably liberated from xylan or xylooligomers and presented as acetic acid. However, accumulation of acetic acid resulting from acetyl removal at high pretreatment severity led to acid-catalyzed hydrolysis of polysaccharide and degradation of monomeric sugars during LHW pretreatment.

The effects of pretreatment time and temperature on the formation of acetic acid during LHW pretreatment are shown in figure 3a. At each temperature, the acetic acid concentrations in 3 and 5 min LHW pretreated liquor were very similar, while significantly higher acetic acid concentrations were observed in 10 min LHW pretreated liquor ($p < 0.05$). Among all pretreatment times tested, the acetic acid concentration increased significantly with temperature ($p < 0.05$). When pretreatment temperature increased from 170 °C to 210 °C, the acetic acid concentration increased from 2.9 to 7.1 g L⁻¹ for a pretreatment time of 10 min, but from 1.9 to 5.3 g L⁻¹ for 3 min, and from 1.7 to 6.1 g L⁻¹ for 5 min. These results indicated that a drastic pH drop resulted from the increase in temperature. During LHW pretreatment of wood sawdust, a pH drop from 5.0 to 2.8 was observed when the temperature was increased from 180 °C to 200 °C (Weil et al., 1998b). As indicated in figure 3b, the formation of acetic acid was also

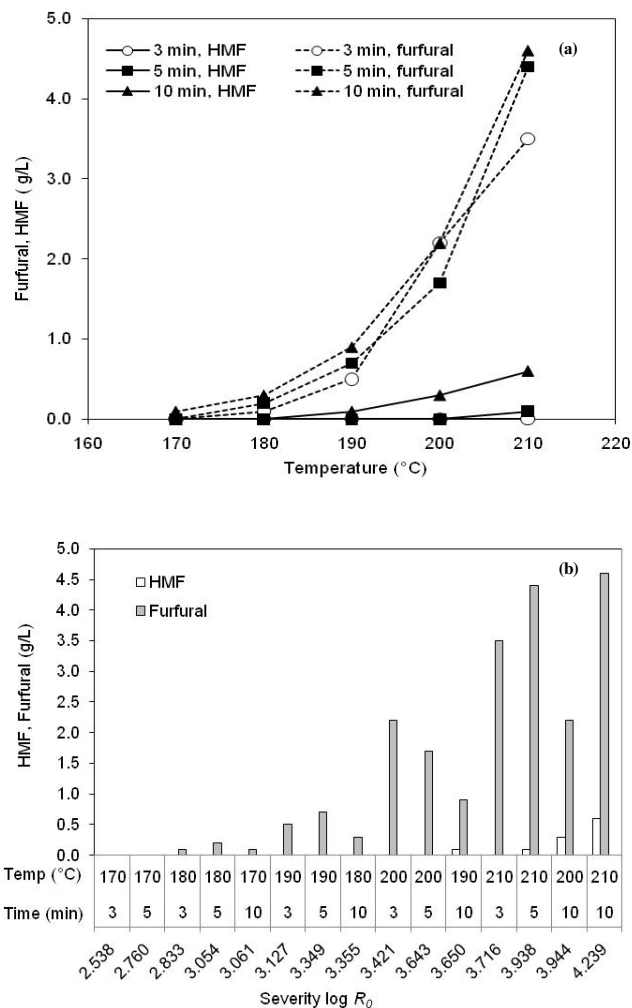


Figure 4. Effect of LHW pretreatment on the formation of HMF and furfural: (a) effect of temperature and time, and (b) effect of severity.

highly correlated to the pretreatment severity, but not as closely as pretreatment temperature.

Formation of HMF and Furfural

The effects of LHW pretreatment on the formation of HMF and furfural are shown in figure 4. Only a low level of HMF (less than 0.6 g L⁻¹) was detected when corn stover was pretreated with LHW at 200 °C and 210 °C for 10 min. HMF was not detected at other pretreatment conditions during this study, which is in agreement with the low cellulose solubilization mentioned previously. The furfural concentration substantially increased with the increase in pretreatment temperature (fig. 4a) but was not closely correlated to pretreatment severity (fig. 4b), indicating that pretreatment temperature played a more critical role in the formation of furfural. When the pretreatment temperature was less than 190 °C, the furfural concentration was less than 1.0 g L⁻¹. The highest furfural concentration (4.6 g L⁻¹) was obtained at pretreatment severity of 4.239 (210 °C, 10 min). In the study of LHW pretreatment of hybrid poplar at uncontrolled pH, increases in furfural concentrations of 1 to 5 g L⁻¹ were reported when temperatures were elevated from 200 °C to 210 °C with pretreatment times of 5 to 20 min (Kim et al., 2009). Although high pretreatment severity is required for effective LHW pretreatment, it is detrimental to monomeric sugar re-

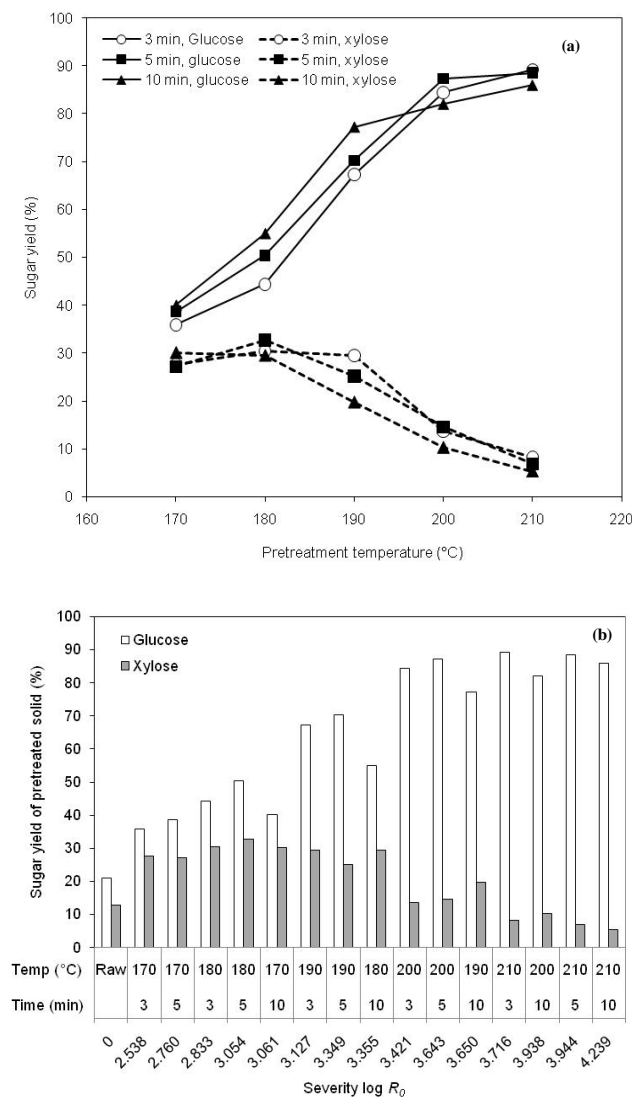


Figure 5. Enzymatic sugar yield of LHW pretreated corn stover: (a) effect of temperature and time, and (b) effect of severity.

covery in the pretreatment liquors, as higher degradation of monomeric sugars occurs at higher severity (Weil et al., 1998b). As furfural at 2 g L⁻¹ or higher is significantly toxic to yeast fermentation (Sanchez and Bautista, 1988; Couallier et al., 2006), it has been suggested that HMF and furfural levels be limited to below 1.0 g L⁻¹ in order to minimize their toxicity to ethanol-producing bacteria and yeast (Kim et al., 2009). Our results showed that a pretreatment temperature of 200 °C for 5 min appeared to be optimal for LHW pretreatment in terms of both cellulose digestibility (fig. 5a) and formation of HMF and furfural.

ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis was conducted to evaluate the effect of LHW pretreatment on the saccharification yield of the LHW pretreated corn stover. As shown in figure 5b, pretreatment at the lower severity 2.538 to 3.054 (170 °C, 3 to 10 min) resulted in a 15% to 18% increase in glucose yield over the control (21.4%). Glucose yield substantially increased with temperature, and the highest glucose yield of 89.2% was obtained at a pretreatment temperature of 210 °C and a pretreatment time of 3 min. The pretreatment severity at this

condition was 3.716. Due to the degradation of sugars, a glucose yield of 85.9% was obtained at the highest pretreatment severity of 4.239 (210 °C, 10 min). Pretreatment time did not significantly affect the glucose yield ($p > 0.05$). For compressed hot water pretreatment of corn stover without pH control in a batch tube reactor instead of a Parr reactor, a glucose yield of 83% based on pretreated corn stover was obtained at the highest pretreatment severity of 4.68 (220 °C, 20 min) (Yang and Wyman, 2004). Yang and Wyman (2004) also reported that no significant increase in glucose yield was observed when the pretreatment severity was increased from 2.50 to 4.68 (160 to 220 °C, 20 min). In contrast, Mosier et al. (2005a) reported that cellulose digestibility of 90% was obtained when corn stover was pretreated at optimized conditions (190 °C for 15 min) with pH control. However, our sugar yields at 190 °C for 3 to 10 min were only 67.3% to 77.2%. The difference of sugar yields between this study and that of Mosier et al. (2005a) could be mainly attributed to the pretreatment conditions (e.g., pH control), reactor configuration, and enzymatic hydrolysis conditions.

During LHW pretreatment, xylan was more liable to be soluble in the liquid fraction of the pretreated materials (fig. 2). Substantial xylan removal also contributed to the improvement of cellulose digestibility. When the soluble xylose in the liquid fraction of the pretreated corn stover was not considered, the xylose yield based on raw material decreased with the increase in pretreatment temperature (fig. 5a). The highest xylose yield of 32.7% was obtained at a pretreatment temperature of 180 °C and a pretreatment time of 5 min. Similar to the effect of LHW pretreatment on the degradation of corn stover, enzymatic hydrolysis was more dependent on pretreatment temperature than pretreatment severity.

CONCLUSION

LHW pretreatment without pH control resulted in 12% to 37% solid solubilization depending on the pretreatment severity (2.538 to 4.239). Cellulose solubilization ranged from 2% to 4% observed when the corn stover was pretreated at 210 °C for 3 to 10 min, while no cellulose degradation was observed at other pretreatment conditions. Xylan solubilization increased with pretreatment temperature and time. More than 90% of the xylan was solubilized when corn stover was pretreated at 210 °C for 10 min. When the pretreatment severity increased from 2.538 to 4.239, the acetic acid concentration increased from 1.9 to 7.1 g L⁻¹. Furfural levels increased substantially with pretreatment temperature, with more than 2 g L⁻¹ of furfural formed at pretreatment temperatures over 200 °C. Significant amounts of HMF were generated when corn stover was pretreated at 200 °C and 210 °C for 10 min. The highest glucose yield of 89.2% was obtained at a temperature of 210 °C and pretreatment time of 3 min. Increasing pretreatment time from 3 to 10 min at this temperature caused decrease in glucose yield.

There was a linear relationship between pretreatment severity and xylan solubilization. The formation of inhibitors and sugar yield were highly dependent on the pretreatment temperature, but they were not well correlated to the pretreatment severity. Thus, the pretreatment severity has limitations for determining the combined effects of both pretreatment temperature and time.

ACKNOWLEDGEMENTS

This work was supported by funding from the OARDC Seeds Program. The authors wish to thank Mrs. Mary Wicks (Department of Food, Agricultural and Biological Engineering, The Ohio State University) for reading through the manuscript and providing useful suggestions.

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